FUJIFILM Wako Pure Chemical Corporation
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Code No. 012-28521 (20 µL)
018-28523 (100 µL)

# Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant

| Code No. | Product name | Pkg. size | Storage |
|---|---|---|---|
| 012-28521 | Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant | 20 µL | -20°C |
| 018-28523 | | 100 µL | |

## Product Summary

| | |
|---|---|
| Reactivity | Mouse, rat (not studied in other species) |
| Clonality | Monoclonal (Clone No. 6A4) |
| Host | Rabbit |
| Conjugate | Unconjugated |
| Concentration | Described on the label |

## Product Details

| | |
|---|---|
| Source | CHO-Spica cell derived antibody |
| Immunogen | Synthetic peptide corresponding to the C-terminus of Iba1 |
| Purity | Protein A affinity chromatography |
| Isotype | IgG |
| Form | Liquid |
| Preservative | 0.05% Sodium azide |
| Buffer | PBS with 50% glycerol |

## Application

Immunohistochemistry (frozen section) 1:200 - 10,000

* The optimal dilutions/concentrations should be determined by each laboratory.

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## Recommend Protocol

### Step 1. Tissue preparation

1. The animal (mouse or rat) should be fixed by perfusing with 4% paraformaldehyde-PBS under the deep anesthesia.
2. After dissection the tissue, sink the tissue in 4% paraformaldehyde-PBS for post-fixation, following to 30% sucrose/ 4% paraformaldehyde-PBS, respectively.
   ∗Sink the tissue until falling-down on the bottom of tube.
3. Mount the tissue with sectioning compound and freeze it completely.
4. Prepare a 20-50 μm thick of frozen section using the cryostat and mount the slice on the pre-coated slide glass for IHC.

### Step 2. Blocking and Permeabilization

1. Rinse the section several times with PBS for removing the sectioning compound.
2. Block the section by Blocking solution (1% animal serum, 2% BSA and 0.3% TritonX-100 in PBS) for 2 hours at room temperature.

### Step 3. Antibody staining

1. Incubate the section with primary antibody solution (Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant, 1000 times dilution in Blocking solution) at 4℃ over night.
2. Wash the section three times with 0.3% TritonX-100 in PBS for 5 min.
3. Incubate the section with secondary antibody solution (i.e. fluorescent dye-conjugated anti-rabbit IgG, appropriatedilution in Blocking solution) for 2 hours RT.
4. Wash the section three times with 0.3% TritonX-100 in PBS for 5 min.

### Optional: Double or nuclear staining

(a) Double staining

· Prepare the different primary and secondary antibodies than those used in “Step 3” and repeat “Step 3”.
  ∗You must prepare a source of primary antibody that is different from the one used in “Antibody staining”.

(b) Nuclear stain

· Prepare DNA binding dye such as DAPI (i.e. code No. 342-07431). The staining protocol follows instruction manual of each dye.

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## Step 4. Mounting and Detection

1. Rinse the section with ddH2O quickly, and snap-off the slide.
2. Absorb remaining ddH2O with paper and drop anti-fade mounding medium, then place a coverslip on the section.
3. Observe the section image by fluorescent microscopy or confocal microscopy with appropriate filter set.